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THE FERTILITY OF ESCHERICHIA COLI ANTIGEN TEST STRAINS IN CROSSES WITH K 12

By

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Sexual recombination in *Escherichia coli* has been studied almost exclusively with strain "K 12". The initial choice of this strain was entirely fortuitous (*Lederberg & Lederberg* 1956, *Gray & Tatum* 1944). While many aspects of the life cycle have been successfully analyzed with this particular strain, at least two considerations have motivated a search for the distribution of sexual interfertility among a wider group of strains: (1) the interest in the status of the *Escherichia coli* group, as a gene pool and (2) the possibility that some genetic differences not evident from mutation in the laboratory might be found among diverse strains from natural habitats. In the latter category, the most interesting features might well involve the mechanism of sexual compatibility among various serotypes.

A preliminary screening of wild type strains seemed to justify these expectations (*Lederberg* 1951). This study was however conducted before the Hfr, F^+, F^- system of sexual compatibility was adequately understood (*Cavalli* 1950, *Lederberg, Cavalli & Lederberg* 1953, *Hayes* 1953). In view of the promise of immunogenetic factors in future work it was, therefore, decided to take advantage of the collection of established serotypes of *Escherichia coli* for a review of genetic interactions in the group. Strain "K 12" itself poses formidable difficulties for immunological studies in account of its virtual loss of O and K antigens, the strain having been cultivated for other purposes and without special scrutiny for its serological properties since 1922. The establishment of a serological scheme for *E. coli* (*Kauffmann* 1954; *Ewing* 1956) indeed represents a substantial investment that can be exploited in further genotypic analysis of the species.

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The E. coli Antigenic Scheme.

Three main types of antigens are depicted in the *E. coli* group: (1) The O antigens which are thermostable lipopolysaccharide-complexes constituting part of the cell wall, (2) the K antigens which are envelope or capsule antigens of polysaccharide nature; historically three types of K antigens A, B and L have been described. They are differentiated by the varying thermostability of their agglutinability, their capacity to evoke the formation of agglutinins and their agglutinin-binding capacity. (3) The H- or flagellar antigens; by contrast with *Salmonella*, which frequently display alternative flagellar forms only monophasic strains have been found so far in the coli group. The first antigenic scheme for the *E. coli* group was published by *Kauffmann* in 1944. This scheme contained 20 different O groups, 17 K antigens and 3 H antigens. Since then the scheme has been steadily extended and it comprised when the present examinations were carried out

137 O antigens
80 K antigens
43 H antigens

corresponding to a somewhat smaller number of type strains, since some of the K and H type strains are also represented in the O series. The serotype of a coli strain is given as follows: O 111:K 58:H 2[1]. The O, K and H antigens exist in a great number of different combinations; if they could recombine freely the absolute number of coli serotypes containing the known antigens would be close to half a million. In practice the number is reduced, as it has been observed that a number of O and K antigens (especially OA and OB) are closely connected. For example it could be mentioned that the combinations O 55:K 59 and O 111:K 58 are known only in the indicated pairs. On the other hand that the H antigens can be combined freely with diverse OK groups is illustrated by the experience from the O 55:K 59 and O 111:K 58 groups. Among the rather limited number of these strains whose H antigens have been recorded at least 8 different H antigen combinations for O 111:K 58 and 10 for O 55:K 59 have been detected (*Ewing* 1956, *Ørskov* 1956).

Sexual Compatibility in E. coli.

Sexual recombination in *E. coli* is mediated by contact between cells of different mating type. In order to give a productive cross one type of cells (F^+) acts as genetic donor or male and the other as recipient or female (F^-). However, F^+ are ambivalent and $F^+ \times F^+$ crosses give a few recombinants. Most of the "K 12" strains are F^+: upon mixing with "K 12" F^- culture they exhibit a low frequency of recombination

[1] According to earlier convention this would have been designated O111:B4:H2; the notations here follows *Kauffmann, Ørskov & Ewing* (1956).

(10^{-5} or less expressed as the observed ratio of recombinant to input parental cells). Strains termed Hfr (*Cavalli* 1950, *Hayes* 1953) show a high frequency of recombination in crosses with F^- strains (10^{-1} to 10^{-3}). In most sexual crossing experiments carried out so far both parent strains have been auxotrophic mutants which were unable to synthesise one or more compounds necessary for growth, most often amino acids. This procedure requires considerable handling of each parent strain to produce the necessary mutants. To simplify the screening of a large number of strains the so-called SRP-technique (*s*treptomycin-*r*esistance-*p*rototroph) was developed (*Lederberg* 1951). Recombinants are selected from test crosses between auxotrophic, streptomycin-resistant tester strains and various prototrophic, streptomycin-sensitive *i.e.* wild type strains. Thus a large number of coli wild type strains could be screened both for fertility and for mating type.

METHODS

The parent strains were grown in penassay broth Difco for 20 hours and 0.5 ml from each parent culture was inoculated into a fresh broth. The mixed culture was incubated for another 20 hours, centrifuged and the pellet resuspended in 0.5 ml distilled water. One drop of this suspension was spread onto minimal medium (see below). Controls were provided by plating the washed parent cultures onto the same medium as used for crosses. The plates were read after 48 hours incubation at 37° C. No further analysis of the recombinant colonies was carried out. (*Lederberg* 1951).

Media. For fluid medium penassay broth Difco was most often used. In some cases autoclaved ox heart infusion broth, produced in Statens Seruminstitut, Copenhagen, was employed. No difference as to the usefulness of these two media could be found. EMS agar plates (*Lederberg* 1950) supplemented with dihydrostreptomycin 100 µg/ml and galactose 1 per cent was used as selective medium for the recombinants.

Strains. All type strains for coli antigens which were established up till 1957 were examined:

O antigen test strains: 137
K antigen test strains: 80
H antigen test strains: 43

Two of the O type strains were found to be streptomycin resistant (O126 and O127), four O type strains have previously been found not to belong to *E. coli* but to the *Citrobacter* group (*Ørskov 1956*) and finally O47 has been lost many years ago. Of the K type strains, 29 were identical with different O type strains. Among the 43 H antigen type strains 23 were already represented among the O or K type strains; two H type strains have been found to belong the *Citrobacter* group. The reduced number of different type strains examined was therefore:

O antigen test strains: 130
K antigen test strains: 51
H antigen test sttrains: 18

Total: 199

76 *E. coli* strains belonging to the OK group O26: K60, O55: K59 and O111: K58, in many different H antigen combinations, were further examined. (Table 1).

72 *Klebsiella* type strains representing the same number of different capsule antigens were also included.

The auxotrophic coli strains with known fertility used in the crosses can be found in Table 2. These last mentioned strains were all developed in Dept. of Genetics, University of Wisconsin, Madison, Wisconsin, U.S.A.

TABLE 1

E. coli Strains Belonging to the OK-Types O26: K60, O55: K59 and O111: K58 Tested.

Serotype antigens O K H	Number of strains
26:60:–	7
26:60:8	1
26:60:11	5
26:60:32	3
55:59:–	8
55:59:2	3
55:59:4	2
55:59:6	9
55:59:7	4
55:59:8	3
55:59:10	2
55:59:11	2
55:59:16	1
55:59:21	1
55:59:25	1
55:59:27	3
55:59:32	3
55:59:34	1
111:58:–	6
111:58:2	4
111:58:4	2
111:58:11	1
111:58:12	1
111:58:16	1
111:58:21	1
111:58:27	1
Totally	76

TABLE 2

Auxotrophic Strains Used.

Source	Strain designation	Markers		Serotype
K12	W1607	F^-	M^-Sr	O^-: K?: H48
–	W3287	Hfr_{13}	M^-Sr	–
WG4	W3703	Hfr	L^-Tryp^-Sr	O25: K^-: nm
H509a	W3479	F^-	H^-Isol^-Sr	O100: K^+: H2
–	W3482	F^+	H^-Isol^-Sr	–

Markers without relation to the study have been omitted. M = methionine, L = leucine, Tryp = tryptophane, H = histidine, Isol = isoleucine and Sr = streptomycin resistant.

W 3287 is an Hfr strain isolated from K12 $F^+M^-Lac^+$ by *J. Lederberg* using indirect selection (replica plating UV-survivors against a Lac^- indicator). Streptomycin resistance was introduced by selection of a spontaneous mutant on streptomycin medium. The K12 strains are characterized as rough, no O antigen has been found till now. The presence of an ordinary K antigen is doubtful. Th H antigen is numbered H48 (*Ørskov & Ørskov* 1960b).

WG4 was isolated in 1950 from a urine culture submitted to the Wisconsin Public Health Laboratory. The markers L^-, $Tryp^-$ and Sr were introduced by conventional

methods (*Lederberg* 1950). After having been converted to the F⁺ state an Hfr mutant W3703 was isolated by the authors using indirect selection against a histidine-less indicator. W3703 has O antigen 25, no detectable K antigen and is non-motile (non-flagellate, therefore missing H antigen).

H509a is the type strain of *E. coli* O antigen 100. The markers H⁻, Isol⁻ and Sr were introduced by conventional methods. W3482 received its F factor from the K12 strain W1876 F⁺ (*Ørskov* & *Ørskov* 1960a). W3479 and W3482 have O antigen O100, a K antigen not yet numbered, and H antigen 2.

TABLE 3

Crosses between Auxotrophic Hfr, F⁺ and F⁻ Strains and Prototrophic E. coli Antigen Type Strains.

Strain no.	Serotype antigens O K H	W3287 K12 Hfr	W3703 WG4 Hfr	W3482 O100 F⁻	W3479 O100 F⁻	W1607 K12 F⁻
O-type strains.						
U4/41	*4*: 3(*L*): 5	++	−	−		−
Bi623/42	*11*: *10*(*L*): *10*	++	+++	−	−	−
F10018/41	*18*: *76B*:*14*	++++	+±+	++		−
K12a	*17*:*16L*: *18*	++	++	−	−	−
F8188/41	*19*: − : 7	−	++	−	−	−
E47a	*25*: *19L*: 12	++++	+±+	+	−	−
P6a	*32*: . : 19	+++±	+++	−	−	
H304	*34*: . : 10	++	−	−	−	−
E77a	*35*: . : 10	++++	±	+	−	−
H510c	*37*: . : 10	−	±	+++	−	−
F11621/41	*38*: . : 26	++++	++++	++++	−	−
H7	*39*: . : −	+++	−	−	−	−
H316	*40*: . : 4	++++	++++	++		−
H710c	*41*: . : 40	++++	++	±+		−
P11a	*42*: . : 37	++++	+++	+	±	−
U19/41	*51*: . : 24	++	+	±	−	
U20/41	52: . : 10	+++	++++	++++	−	−
Bi7327/41	*53*: . : 3	++++	++	++++	++	
Su3684/41	*56*: . : −	++++	++	−	−	
F8198/41	*57*: . : −	++	++	+++	−	−
F8962/41	*58*: . : 27	+++	±	−	−	++
F10524/41	*62*: . : 30	−	+++	−		−
K6b	*64*: . : −	+++	++	++	−	−
K11a	*65*: . : −	+++	±+	−	−	−
P1a	*66*: . : 25	+++	+++	−	−	−
P9b	*69*: . : *38*	+++	±+	−	−	−
P10a	*71*: . : 12	++++	+++	−	−	−
P12a	*73*: . : 31	+++	+++	−		−
E3a	*74*: . : 39	+++	++++	++		+
E5d	*76*: . : 8	++++	++	−	−	−
E71	*80*: . : 26	+±+	+±+	+	+	−
H19	*84*: . : 21	++++	++	++++	−	−
H35	*86*: . : 25	++	++±	++	+	−
H40	*87*: . : 12	+++±	++++	−	−	−
H68	*89*: . : 16	±+++	+++	++	−	−
H77	*90*: . : −	±±±	−		−	−
H307b	*91*: . : −	+±	−	−	−	−
H308a	*92*: . : 33	−	+++	−	−	−
H319	*96*: . : 19	+++	++		−	−
H504c	*99*: . : 33	+++	−	−	−	−

TABLE 3 (cont.)

Strain no.	Serotype antigens O K H	W3287 K12 Hfr	W3703 WG4 Hfr	W3482 O103 F^+	W3479 O100 F^-	W1607 K12 F^-
O-type strains.						
H509a	*100*: . : 2	++++	+++	++	–	–
H511	*102*: . : 8	++++	+++	++	·	–
H519	*104*: . : 12	++++	+++	++	·	–
H521a	*106*: . : 33	+++	+++	–	–	–
H705	*107*: . : 27	+++	++	·	·	–
H708b	*108*: . : 10	+++	–	+++	–	–
26w	*114*: . : 32	++++	–	++	·	–
28w	*116*: . : 10	+++	+	++	–	–
30w	*117*: . : 4	+++	+	–	–	–
31w	*118*: . : –	+++	++	·	–	–
43w	*123*: . : 16	–	+++	++++	–	–
Canioni	*125*: *70B*: 19	+++	–	++	–	·
178/54	*129*: . : 11	++++	+++	+++	–	–
4866/33	*130*: . : 9	++++	+++	+++	–	–
S239	*131*: . : *26*	+++	–	+	–	–
N87	*132*: . : 28	++	+	++	–	–
4370/53	*134*: . : 35	+++	++	+	–	–
coli Pecs	*135*: . : –	+++	++	+++	–	–
K-type strains not listed above.						
Pus 3422/41	7: 7L: 4	++	+++	+++	–	–
H67	23: *22L*: –	+++	+++	+	·	–
Bi449/42	9a: *26A*: –	+	+++	+	–	–
E56b	8: *27A*: –	+	+++	–	·	–
H36	9: *32A*: 19	–	++	–	–	–
A198a	9: *36A*: 19	–	++	++	–	–
A262a	9: *38A*: –	–	++	–	–	–
A12b	6: *54L*: –	+++	+++	–	–	–
N24c	9: *55A*: –	–	++	–	–	–
5017/53	86ab: *64B*: *36*	++	++	·	–	–
2160/53	127ab: *65B*: 4	++	++	–	–	–
H-type strains not listed above.						
Bi7575/41	8: *25B*: *9*	++	++	–	–	–
H330b	8: . : *20*	++	++	–	–	–
K42	45: . : *23*	–	+	+++	–	–
K72	51: . : *24*	–	+++	–	–	·
K181	11: . : *33*	++	±	–	–	–

±	=	1–2	colonies.
+	=	10	–
++	=	10–50	–
+++	=	50–200	–
++++	=	> 200	–

In the serotype formulas · means, not examined.
– means, antigen not present.
Only productive crosses have been recorded.
The antigen for which the corresponding strain is the official type strain has been written in italics in the serotype column.

EXPERIMENTAL

199 different *E. coli* antigen type strains were crossed with the following strains of known fertility: Two Hfr strains W 3287 and W 3703 coming from the "K 12" and the WG 4 line respectively, further one F^+ strain W 3482 derived from the coli O 100 type strain. Finally crosses with two F^- strains, W 1607 from the "K 12" line, and W 3479 derived from coli O 100 were carried out. The two last mentioned strains were included to see if any F^+ strains were represented among the type strains. All positive crosses were carried out at least twice. The control plates on which the two parent strains were inoculated separatively showed no growth in the recorded cases.

TABLE 4

Crosses between Auxotrophic Hfr, F^+ and F^- Strains and Prototrophic E. coli Strains Isolated from Infantile Diarrhoea.

Strain no.	Serotype antigens O K H	W3287 K12 Hfr	W3482 O100 F+	W3479 O100 F-	W1607 K12 F-	Country where isolated
C24–55	26 : 60 : 32	+++	++		–	Switzerland
C116 55	26 : 60 : 32	+++	+++	–	–	Mexico
C56–56	26 : 60 : 32	+++	±	–	–	Wales
F53–50	55 : 59 : 2	+++		±	–	Sweden
C572–54	55 : 59 : 2	++++	–	–	–	Germany
C218-54	55 : 59 : 10	++++	+	–	–	
C50–56	55 : 59 : 11	++++	++	–	–	Wales
C222–53	55 : 59 : 11	+++	+		–	France
C223–53	55 : 59 : 21	+++	++	–	–	
C238–56	55 : 59 : 25	+++	+	–	–	
C87–53	111 : 58 : –	+++	+	–	–	

± = 1–2 colonies.
\+ = 10 –
++ = 10–50 –
+++ = 50–200 –
++++ = > 200 –

In the serotype formulas · means, not examined.
– means, antigen not present.

The outcome of the crosses is shown in Table 3. It appears that 74 different strains were fertile in crosses with W 3287 or W 3703, *i.e.* 37 per cent. Sixtythree strains were fertile with W 3287 and sixtyfour with W 3703. Thirtyseven strains were fertile in crosses with the F^- strain W 3482. Generally there was good agreement between the fertile strains found in the three series of crosses; only 10 strains were fertile with W 3287 and not with W 3703, and 11 strains fertile with W 3703 and not with W 3287. All strains fertile with W 3482 gave productive crosses with either W 3287 or W 3703. Generally the number of recombinants was largest in the W 3287 crosses. Only a few examples of F^+ strains *i.e.* strains giving recombinants in crosses with F^- strains, were detected, and the number of recombinants was low in these cases.

All crosses were carried out at least twice and as could be expected the number of recombinants produced in a given cross could vary to a large extent from time to time.

In addition to the coli type strains further 76 coli strains belonging to the OK groups O 26:K 60, O 55:K 59 and O 111:K 58 in a variety of H antigen combinations were tested (Table 4). These strains were isolated from outbreaks and single cases of infantile diarrhoea and in some cases from healthy or diseased animals. Eleven strains gave productive crosses with W 3287, of these nine could also be crossed with W 3482, but these crosses were less productive. None were fertile with the F^- strains W 3479 and W 1607. It should be pointed out that two strains out of three belonging to O 55:K 59:H 2, and that two out of two belonging to O 55:K 59:H 11 and furthermore three out of three belonging to O 26:K 60:H 32 were fertile in crosses with W 3287. In order to show that there were no direct epidemiological connection between these fertile strains of the same serotype, the place where the strains were originally isolated have been recorded in the table. In order to test if some *Klebsiella* strains were fertile in crosses with W 3287, the 72 *Klebsiella* capsule type strains were examined. Eight were found to be streptomycin resistant and could not be tested with the SRP technique. In none of the remaining cases recombinants could be detected.

DISCUSSION

It appears from the recorded experiments that about one third of two hundred serologically different *E. coli* strains are fertile in crosses with either or both of two Hfr testers having the K 12 fertility factor. This figure is considerably higher than earlier reported figures, (*Lederberg et al.* 1952). As already mentioned Lederbergs screening of the fertility of wild type strains was carried out before the Hfr, F^+ and F^- mating system was adequately understood and an F^+ strain was used as donor instead of the Hfr strain used here. An F^+ strain from the "K 12" line was not included among the donor strains in this study, but even the O 100 F^+ strain, W 3482, seems to detect more fertile coli wild type strains (18 per cent) than the "K 12" F^+ strain used in Lederbergs investigation. One explanation for this discrepancy could be that the criteria for naming a strain *E. coli* perhaps were less strict among the strains in the earlier reported series, than they were among the type strains which never deviated much from the recognized biochemical pattern of *E. coli*; the "K 12" wild type strain will show that pattern without deviations. Another explanation might be the different origin and the different age as laboratory strains of the two series of strains. The strains used by *Lederberg et al.* were mostly freshly isolated strains from pathological conditions, while most of the coli type strains have been kept in the laboratory for many years and only part of them

are from pathological conditions. It is well known that the O and K antigens of freshly isolated strains are bettter developed than in old laboratory strains and further several investigators (*Kauffmann* 1944, *Vahlne* 1945) have shown that the frequency of O-inagglutinable strains, indicating strains with well developed K antigens, is greater among strains from pathological conditions than among strains isolated from normal faeces. It is therefore probable that the strains examined by *Lederberg* had better developed K and O antigens, and further that the readiness with which they mutated to R forms was less than that of the old laboratory strains used in this study. Nobody has yet reported if fertility in *E.coli is* influenced by qualitative and quantitative differences of the O and K antigens, but it may be that strains with poor development of these antigens are more fertile. In this connection it could also be pointed out that none of the 72 *Klebsiella* strains, which all have very large capsules were found to give productive crosses with the Hfr and F^+ strains used here. In another investigation (*Ørskov, Ørskov & Kauffmann* 1961) it was found that more than 50 per cent of randomly selected *Salmonella* strains representing all *Salmonella* O groups were fertile with the "K 12" Hfr strain used in this study; it is known that K antigens if present, are only poorly developed in *Salmonella* strains.

Many of the O antigens of the coli type strains are interrelated, but only in very few cases have these relationships been definitely elucidated *i.e.* the coli antigenic scheme is not so explicitly developed as to give the highly differentiated tabulation of the different O antigen fractions which is characteristic of the Kauffmann-White scheme for the Salmonella group. What does exist is a listing of the O antigen agglutination titres from mutual agglutinations of O test strains in O antigen typing sera (*Ewing* 1956). When such a list is compared with the result of the compatibility examination recorded in this paper, it is difficult to find any relationship between special O antigens or O antigen fractions and fertility.

The K antigens of the examined strains are to a great extent not numbered yet, but from unpublished findings it is known that a large fraction of the unnumbered K antigens from the O series represent new and different K antigens most of them probably B antigens. It is therefore also difficult to find any relationship between compatibility with the testers used and the specificity of the K antigens. When we finally turn to the H antigens it is not possible to find any correlation between compatibility and the different H antigens. One exception is H 10 which is found in 9 out of 130 strains in the O series; 7 of these are fertile with one or more of the male testers.

The outcome of the crosses involving more identical strains of the same serotype seem to tell more of the role of antigenic components in compatibility studies. It appears that neither O nor K antigens alone can determine the compatibility, because only some of the strains with

the O 26:K 60 or O 55:K 59 complex are fertile with the testers used. The results seem to imply that the serotype is of some importance, because more cases were recorded where strains of the same serotype of independant origin were compatible.

The simplest explanation would be that such fertile strains of the same serotype were derived from the same ancestral strain.

With this explanation in consideration we cannot draw any conclusions from the recorded results as to a possible genetical or physiological connection between the antigenic composition of a certain serotype and its fertility.

No large scale examination of the interfertility of the type strains that were found to be fertile in these studies have been carried out. In a limited number of cases such fertile strains, after conversion to the F^+ state could also be crossed with one another (*Ørskov & Ørskov*).

Two thirds of the examined strains were found to be sterile. Future research will show if those strains are completely sterile or if they belong to other independant breeding groups. Finally it should be kept in mind that the sterility detected might disappear when a different crossing technique was employed.

SUMMARY

A screening for fertility of 199 *E. coli* antigenic type strains (O, K and H-antigens), using a number of testers of known fertility showed that about 35 per cent were fertile. With few exceptions the strains were found in the F^- state.

No definite correlation between fertility and single antigens could be found.

A similar screening of a number of coli strains having relations to types found in infantile diarrhoea was performed. In a number of cases there seemed to be some correlation between the serotype and the fertility.

This fact is considered attributable, not to a connection of antigenic structure with fertility but to a common origin of the strains.

Finally all *Klebsiella* capsule type strains were examined; none were found to be fertile.

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